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(54) Title: PHARMACEUTICAL COMPOSITIONS USEFUL FOR THE TREATMENT OF CANCERS

(57) Abstract: The present invention relates to the use of at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives, for the preparation of a drug for the treatment of cancers.

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PHARMACEUTICAL COMPOSITIONS USEFUL FOR THE TREATMENT OF CANCERS

The present invention relates to new pharmaceutical compositions useful for the treatment of cancers.

Cancers are a group of pathologies characterized, in particular, by abnormal cell proliferation. Among the many strategies used to cure cancers, one of them relies on the induction of cell differentiation to halt the multiplication of cancerous cells.

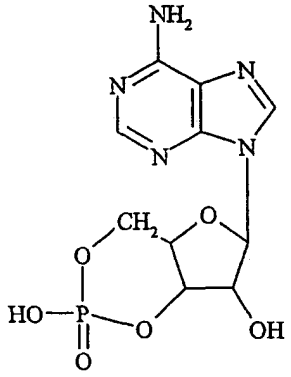
As such, certain leukemia, notably acute myeloid leukemia, are characterized by an arrest in differentiation, induction of proliferation and repression of normal hematopoiesis. These malignancies are often associated to recurrent chromosomal translocations, most of which encode fusion proteins derived from transcription factors (1). Functional analyses of several of these fusion proteins have shown that they behave as potent transcriptional repressors (2), often through modifications of chromatin structure by histone desacetylases, blocking expression of unidentified genes that control myeloid differentiation. Transcription therapy attempts to re-express these genes, resulting in restoration of differentiation. Inhibition of desacetylases by a variety of compounds has shown some efficacy in cell culture (3) and in animal models of leukemia (4), but, as yet, there is only little evidence for their beneficial effects in clinical settings (5). To date, the only real clinical success of transcription/differentiation therapy is acute promyelocytic leukemia (APL), for which two drugs, retinoic acid (RA) and arsenic trioxide (As_2O_3) induce remissions (6, 7). Remarkably, these two drugs target the oncogenic PML/RARα fusion protein and reverse PML/RARα-mediated repression (8). However, in certain cases resistance to both drugs has arisen.

Cyclic AMP (cAMP, adenosine 3'-5' cyclic monophosphate), or its derivatives, could also be viewed as a drug of choice for the induction of differentiation. Indeed, *ex vivo*, activation of the cAMP signal transduction pathway differentiates many acute myeloid leukemia cell-line and strongly synergizes with other differentiating agents ((9-11), reviewed in (12)). Yet, a number of acute toxicities have precluded or severely limited *in vivo* trials using cAMP, or its derivatives (13), and its potential benefits in the treatment of cancers have never been soundly assessed.

Thus, an object of the present invention is to provide new pharmaceutical compositions, comprising at least one compound activating the cAMP signal transduction pathway, useful for the treatment of cancers.

The present invention relates to the use of at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives, for the preparation of a drug for the treatment of cancers.

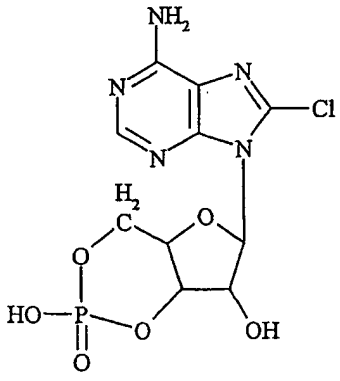
cAMP corresponds to the following formula:



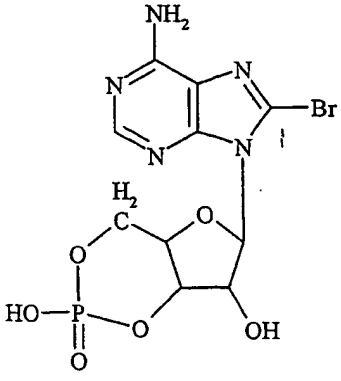
cAMP

The derivatives of cAMP are well known to the man skilled in the art, they notably comprise 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP and dibutyryl-cAMP, or pharmacologically acceptable salts thereof.

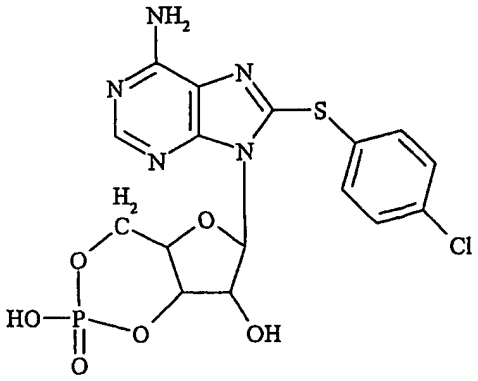
The formulae of several derivatives of cAMP are shown below:



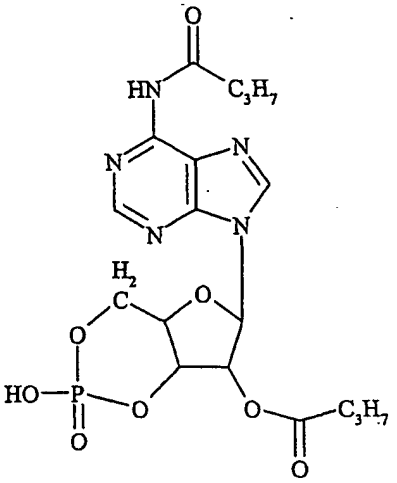
8-Cl-cAMP



8-Br-cAMP



8-CPT-cAMP



dibutyryl-cAMP

The "originally present cellular content of cAMP" relates to the cAMP content of cells prior to the addition to said cells of any compound liable to modify the cellular concentration of cAMP.

The cellular content of cAMP, or of its derivatives, can be measured according to methods well known to the man skilled in the art.

The cAMP content of a cell results from an equilibrium between two opposite reaction types, i.e. reaction concurring to the synthesis of cAMP, such as reactions catalyzed by adenylate cyclases, and reactions concurring to the degradation of cAMP, such as reactions catalyzed by phosphodiesterases (PDE). Consequently, a rise in the cellular content of cAMP can be observed following addition of compounds either activating cAMP synthesis or inhibiting cAMP degradation. Thus, an "agent enabling to increase the cellular content of cAMP or derivatives thereof", can be for instance, cAMP or a derivative thereof in itself, or an agent activating the intracellular synthesis of cAMP, or an agent inhibiting the intracellular degradation of cAMP or derivatives thereof, provided it is added to cells in an amount sufficient to lead to an increase of the cAMP content of said cells.

The present invention also relates to the use of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives and
- at least one cell-differentiation factor or precursors or derivatives thereof and/or
- at least one apoptotic inducer

for the preparation of a drug for the treatment of cancers.

A "cell differentiation factor" refers to compounds liable to induce cellular differentiation, such as retinoic acid, interferons, cytokines or growth factors.

An "apoptotic inducer" refers to compounds liable to induce programmed cell death, such as cancer chemotherapeutic agents or arsenic derivatives (As_2O_3, As_4S_4).

According to an advantageous embodiment, the invention relates to the use of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives and
- at least one cellular differentiating factor or precursors or derivatives thereof

for the preparation of a drug for the treatment of cancers.

Advantageously this association is synergic.

According to another advantageous embodiment, the invention relates to the use of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP and
- at least one apoptotic inducer

for the preparation of a drug for the treatment of cancers.

Advantageously this association is synergic.

According to another advantageous embodiment, the invention relates to the use of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives,
- at least one cellular differentiating factor or precursors or derivatives thereof and
- at least one apoptotic inducer

for the preparation of a drug for the treatment of cancers.

Advantageously this association is synergic.

In an advantageous embodiment of the invention, the agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives is selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof.

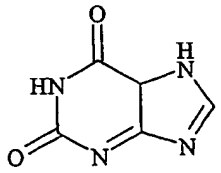
Particular cAMP derivatives can be selected according to their respective properties, well known to the man skilled in the art, such as stability, solubility, efficacity, or toxicity, as compared to cAMP or other cAMP derivatives, for a given use.

The invention relates more particularly to the abovementioned uses, wherein the agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives is a phosphodiesterase inhibitor.

cAMP, and other cyclic nucleotides, are respectively hydrolyzed to AMP, and to the corresponding acyclic nucleotide, by phosphodiesterases (PDE); phosphodiesterase inhibitors limit the hydrolysis of cAMP, and of other cyclic nucleotides, and thus enable to increase the cellular content of cAMP, and of other cyclic nucleotides, as discussed above.

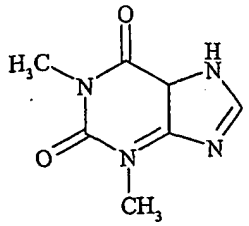
The invention also relates to the preceding use, wherein the phosphodiesterase inhibitor is selected from the group comprising methylxanthines such as caffeine or theophylline or aminophylline or isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, or methoxyquinazoline.

Xanthine corresponds to the following formula:

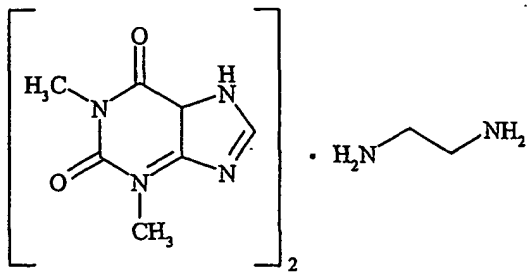


Methylated derivatives of xanthine are phosphodiesterase inhibitors well known to the man skilled in the art and correspond for example to:

- Theophylline (1,3 dimethylxanthine):

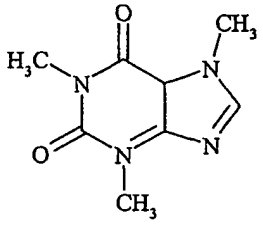


- Aminophylline (1,3 dimethylxanthine complexed to diaminoethan):

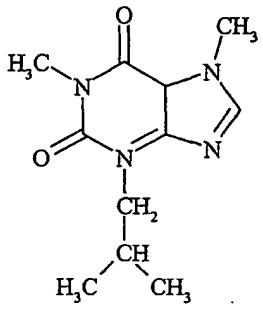


Advantageously aminophylline has an increased solubility as compared to theophylline.

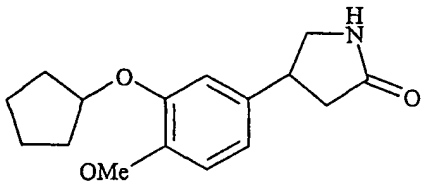
- Caffeine (1,3,7 trimethylxanthine):



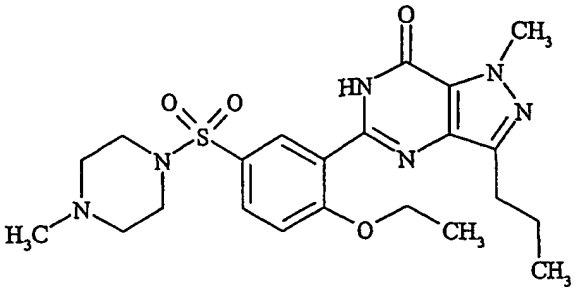
- Isobutyl-methylxanthine (3-isobutyl-1-methylxanthine) :



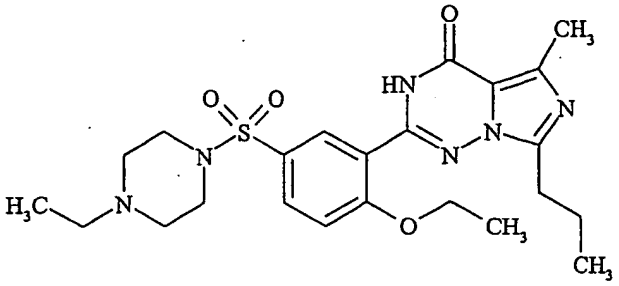
Rolipram, sildenafil (Viagra®), vardenafil, zaprinast, or methoxyquinazoline, are phosphodiesterase inhibitors well known to the man skilled in the art.



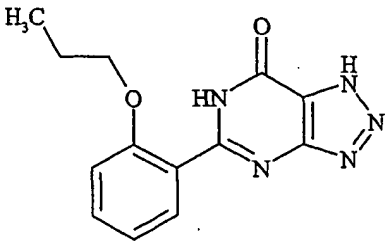
Rolipram



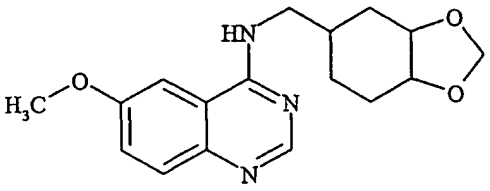
Sildenafil



Vardenafil



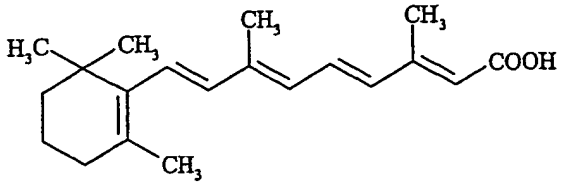
Zaprinast



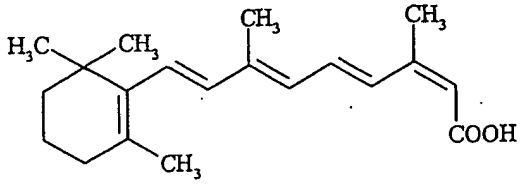
methoxyquinazoline (MQZ)

The invention more particularly relates the above mentioned use, wherein the phosphodiesterase inhibitor is theophylline or aminophylline.

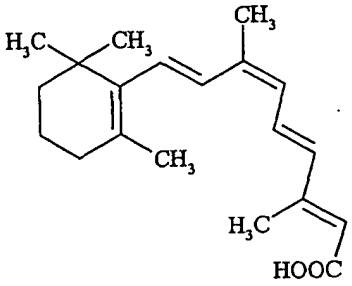
According to yet another embodiment, the invention relates to the preceding uses wherein the cell-differentiation factor or precursors or derivatives thereof is selected from the group comprising retinoic acid, particularly all-trans retinoic acid or 9-cis retinoic acid or 13-cis retinoic acid, or pharmacologically acceptable salts thereof, vitamin A (retinol), carotene or rexinoids.



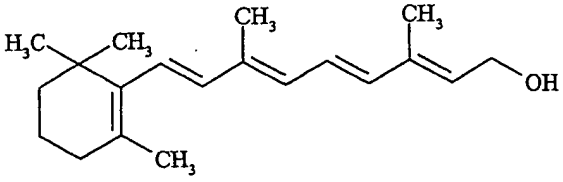
All-trans retinoic acid



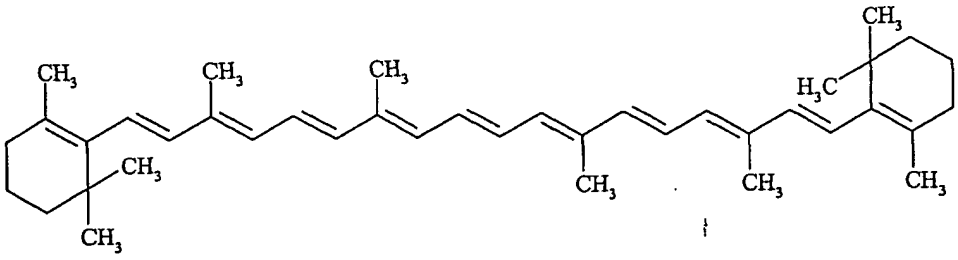
13-cis retinoic acid



9-cis retinoic acid

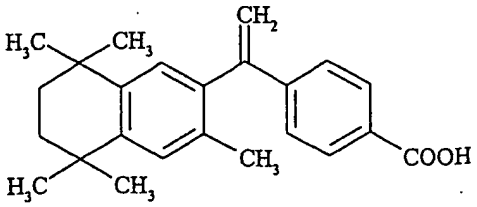


Retinol (all-trans)

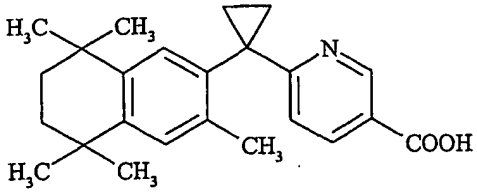


Carotene (β configuration)

Rexinoids are specific ligands of the RXR receptors, they notably comprise LG 100268 (LG268) or LGD 1068 (Targretin) for example.



LGD 1068



LG268

The invention also relates to the abovementioned use wherein the cell-differentiation factor or precursors or derivatives thereof is retinoic acid, particularly all-trans retinoic acid, or pharmacologically acceptable salts thereof.

According to another particular embodiment, the invention relates to the above mentioned use, wherein the apoptotic inducer is selected from the group comprising arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

Arsenic trioxide is notably described in Chen *et al.* (1996) *Blood* **88**:1052-1061.

Arsenic sulfide is notably described in Lu *et al.* (2002) *Blood* **99**:3136-3143.

Both compounds have similar properties and act on similar cellular targets.

According to still another particular embodiment, the invention relates to the above mentioned use of theophylline or aminophylline and retinoic acid or pharmacologically acceptable salts thereof, for the preparation of a drug for the treatment of cancers.

Advantageously theophylline, or aminophylline, synergizes with retinoic acid.

The present invention also relates to the abovementioned use of theophylline or aminophylline and arsenic trioxide or arsenic sulfide, for the preparation of a drug for the treatment of cancers.

Advantageously theophylline, or aminophylline, synergizes with arsenic trioxide, or arsenic sulfide.

The present invention equally relates to the abovementioned use of theophylline or aminophylline, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, for the preparation of a drug for the treatment of cancers.

Advantageously the association of theophylline, or aminophylline, and retinoic acid, and arsenic trioxide, or arsenic sulfide, is synergic.

According to a further embodiment, the invention relates in particular to abovementioned uses, wherein the cancers are selected from the group comprising solid tumor cancer, neuroblastoma, skin cancer, oral cavity cancer, lung cancer, mammary gland cancer, prostatic cancer, bladder cancer, liver cancer, pancreatic cancer, cervical cancer, ovarian cancer, head and neck cancer, colon cancer, germ cell cancer, leukemia, acute leukemia, acute myelocytic leukemia, acute promyelocytic leukemia, aleukemic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia.

According to another aspect, the invention relates to products containing 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

The invention relates in particular to products as defined above, containing 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

The invention more particularly relates to the above defined products, containing 8-Cl-cAMP or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

The invention further relates to the abovementioned products, containing 8-Cl-cAMP or pharmacologically acceptable salts thereof, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

According to another embodiment, the invention relates to products containing aminophylline or theophylline and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

The invention relates in particular to products as defined above, containing aminophylline or theophylline and retinoic acid or pharmacologically acceptable salts thereof, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

The invention also relates to products as defined above, containing aminophylline or theophylline and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

Advantageously, the invention relates to products as defined above, containing aminophylline or theophylline, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

According to yet another aspect, the present invention also relates to a pharmacological composition comprising as active substance 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically

acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

The invention relates in particular to a pharmacological composition as defined above, wherein the active substance is 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof, in association with a pharmacologically acceptable vehicle.

The invention also relates to a pharmacological composition as defined above, wherein the active substance is 8-Cl-cAMP or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

The invention further relates to a pharmacological composition as defined above, wherein the active substance is 8-Cl-cAMP or pharmacologically acceptable salts thereof, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

The present invention also relates to a pharmacological composition comprising as active substance theophylline or aminophylline and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

The invention relates in particular to a pharmacological composition as precedingly defined, wherein the active substance is theophylline or aminophylline and retinoic acid or pharmacologically acceptable salts thereof, in association with a pharmacologically acceptable vehicle.

Advantageously the invention relates to a pharmacological composition as defined above, wherein the active substance is theophylline or aminophylline and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

The invention also relates to an abovementioned pharmacological composition, wherein the active substance is theophylline or aminophylline, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

The invention relates in particular to a pharmacological composition as defined above, in a form appropriate for the administration of about 0.36 mg/kg/day to about 14.3 mg/kg/day of theophylline or aminophylline, of about 4.5 mg/m^2/day to about 135 mg/m^2/day of all-trans retinoic acid and of about 0.014 mg/kg/day to about 0.43 mg/kg/day of arsenic trioxide.

Brief description of the figures

Figure 1A, Figure 1B, Figure 1C, Figure 1D, Figure 1E, Figure 1F, Figure 1G, Figure 1H, Figure 1I, Figure 1J

Figure 1A represents the spleen weight (vertical axis, mg) of retinoic acid sensitive mice treated (+) or untreated (-) by 8-Cl-cAMP during 7 days.

Figure 1B represents the spleen weight (vertical axis, mg) of retinoic acid sensitive mice treated (+) or untreated (-) by 8-Cl-cAMP during 7 days.

Figures 1C, 1D, 1E et 1F represent pictures of bone marrow samples, after May-Grünwald-Giemsa (MGG) staining, taken from retinoic acid sensitive mice treated (figure 1D) or untreated (figure 1E) by 8-Cl-cAMP and from retinoic acid resistant mice treated (figure 1F) or untreated (figure 1G) by 8-Cl-cAMP.

Figures 1G, 1H, 1I et 1J represent pictures of liver samples, after hematoxylin-eosin staining, taken from retinoic acid sensitive mice treated (figure 1G) or untreated (figure 1H) by 8-Cl-cAMP and from retinoic acid resistant mice treated (figure 1I) or untreated (figure 1J) by 8-Cl-cAMP.

In figure 1F an apoptotic cell is marked by an arrow.

Figure 2A, Figure 2B, Figure 2C, Figure 2D, Figure 2E, Figure 2F

Figure 2A represents the spleen weight (vertical axis, mg) of a retinoic acid sensitive mouse model of APL treated during 3 days by 8-Cl-cAMP (cAMP), As_2O_3 (As), 8-Cl-cAMP and As_2O_3 (cAMP + As), or untreated (Ø).

Figure 2B represents a western blot of protein extracts of bone of retinoic acid sensitive APL mice revealed by an anti-p21 antibody (arrow). The APL mice were either treated 24 h *in vivo* by retinoic acid (RA), As_2O_3 (As), 8-Cl-cAMP (cAMP), or untreated (Ø). The star (*) denotes to a cross-reactive protein.

Figure 2C represents a picture of a bone marrow sample, after May-Grünwald-Giemsa (MGG) staining, taken from retinoic acid sensitive APL mice treated by 8-Cl-cAMP (cAMP), As_2O_3 (As), 8-Cl-cAMP and As_2O_3 (cAMP + As), during 3 days, or untreated (Ø).

Figure 2D represents a picture of a liver sample, after hematoxylin-eosin staining, taken from retinoic acid sensitive APL mice treated by 8-Cl-cAMP (cAMP), As_2O_3 (As), 8-Cl-cAMP and As_2O_3 (cAMP + As), during 3 days, or untreated (Ø).

Figure 2E represents a picture of a bone marrow sample, after May-Grünwald-Giemsa (MGG) staining, taken from retinoic acid sensitive APL mice treated by theophylline (T), As_2O_3 (As), theophylline and As_2O_3 (T + As), during 3 days, or untreated (Ø).

Figure 2F represents the percentage of NBT positive NB4 cells (vertical axis) treated by As_2O_3 at various concentrations (from left to right) 0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M, in the presence (+) or the absence (-) of 8-Cl-cAMP.

Figure 3A, Figure 3B, Figure 3C, Figure 3D

Figure 3A represents the spleen weight (vertical axis, mg) of a retinoic acid sensitive mouse model of APL treated during 7 days by retinoic acid (RA), 8-Cl-cAMP (cAMP), retinoic acid and 8-Cl-cAMP (RA + cAMP), or untreated (Ø).

Figure 3B represents a picture of a bone marrow sample, after May-Grünwald-Giemsa (MGG) staining, taken from retinoic acid sensitive APL mice treated by retinoic acid during 7 days.

Figure 3C represents a picture of a bone marrow sample, after May-Grünwald-Giemsa (MGG) staining, taken from retinoic acid sensitive APL mice treated by retinoic acid and 8-Cl-cAMP during 7 days.

Figure 3D represents the number of bone marrow blasts (horizontal axis) marked by an anti-CD11 antibody (vertical axis) of APL mice treated for 24 hours by cAMP (cAMP), As_2O_3 (As), 8-Cl-cAMP and As_2O_3 (As + cAMP) or untreated (0).

Figure 4A, Figure 4B, Figure 4C, Figure 4D, Figure 4E, Figure 4F, Figure 4G, Figure 4H, Figure 4I, Figure 4J

Figure 4A represents the spleen weight (vertical axis, mg) of a retinoic acid resistant mouse model of APL treated during 7 days by 8-Cl-cAMP (cAMP), As_2O_3 (As), retinoic acid (RA), retinoic acid and 8-Cl-cAMP (RA + cAMP), As_2O_3 and 8-Cl-cAMP (As + cAMP), retinoic acid and As_2O_3 (RA + As), or untreated (Ø).

Figures 4B, 4C, 4D, 4E, 4F, 4G, 4H, 4I, 4J represent pictures of bone marrow samples, after May-Grünwald-Giemsa (MGG) staining, taken from retinoic acid resistant APL mice treated by 8-Cl-cAMP (Figure 4C), As_2O_3 (Figure 4E), retinoic acid (Figure 4D), retinoic acid and 8-Cl-cAMP (Figure 4F, 4G), As_2O_3 and 8-Cl-cAMP (Figure 4H), retinoic acid and As_2O_3 (Figure 4I), or untreated (Figure 4B).

Figure 5A, Figure 5B, Figure 5C, Figure 5D, Figure 5E, Figure 5F, Figure 5G

Figures 5A, 5B and 5C represent pictures of bone marrow samples taken at day 0 (figure 5A), at day 14 (figure 5B) or at day 28 (figure 5C) from a retinoic acid/As2O3 resistant APL patient treated by a combined retinoic acid/As2O3 therapy.

Figures 5D, 5E and 5F represent pictures of bone marrow samples taken at day 0 (figure 5D), at day 14 (figure 5E) or at day 28 (figure 5F) from the same retinoic acid/As2O3 resistant APL patient treated by a combined retinoic acid/As2O3/theophylline therapy.

Figure 5G is a schematic representation of the clinical events of the treatment of a patient by a combined theophylline/As2O3/RA therapy. The horizontal axis represents the time course in weeks. The left upward vertical axis and the corresponding curves represent hemoglobin concentration (Hb) in gr/l and the platelet (Plt) count per mm^3 (times 10^3), the right upward vertical axis and the corresponding curve represent the white blood cells (WBC) count per mm^3 (times 10^3). The left downward vertical axis and the left bars (dark gray) represent the blast count in percent, the right downward vertical axis and the right bars (light gray) represent the erythroblast count in percent. The upper downward arrows represent red cells transfusions and the lower downward arrows represent platelet transfusions. The three upper horizontal black bars at the bottom of the figure represent the periods of combined As2O3/all-trans retinoic acid treatment (As_2O_3/ATRA), the lower horizontal black bar at the bottom represent the period of theophylline treatment.

EXAMPLES

EXAMPLE 1

cAMP synergizes with As_2O_3 to differentiate APL cells ex vivo

cAMP is well-known to greatly enhance RA-induced (retinoic acid) differentiation of many cell lines derived from embryonal carcinoma or myeloid leukemia, in particular APL (acute promyelocytic leukemia) (11). Low concentrations of As_2O_3 can induce incomplete differentiation in an APL cell line (7). The Inventors tested the hypothesis that cAMP would also enhance As_2O_3-induced differentiation.

The APL model cell line NB4 was cultured as described previously in Lanotte *et al.* (1991) *Blood* 77:1080-1086. Morphology and cellular differentiation were evaluated on May-Grünwald-Giemsa-stained cytospins. Differentiation was quantified by reduction of nitroblue-tetrazolium (NBT) according to procedure well known to the man skilled in the art. 8-Cl-cAMP (8-Chloro-adenosine 3-5' cyclic monophosphate) and 8-CPT-cAMP (8-(4-chlorophenylthio)adenosine 3-5' cyclic monophosphate (15), a low toxicity cAMP analogue) were obtained from Biolog Life Research Institute (Bremen, Germany) and Sigma (St. Louis, MI), respectively. 8-CPT-cAMP was used at a concentration of 2.10^{-4}M.

As shown in **Figure 2F**, even very low doses of As_2O_3 (Sigma) combined with 8-CPT-cAMP induced NBT reduction in 40% of the cells, while cAMP or As_2O_3 alone had insignificant effects. Higher As_2O_3 concentrations inhibited NBT reduction, as reported. Similarly, only combined As_2O_3 and 8-CPT-cAMP induced morphological differentiation into myelocyte-like cells, consistent with a very recent report (17).

EXAMPLE 2

Antileukaemic effects of cAMP in two models of APL mice

To assess a possible *in vivo* efficiency of cAMP, the Inventors turned to a transplantation APL model (14) derived from RA-sensitive *PML/RARα* transgenics (18). The Inventors similarly developed a transplantation model for RA-resistant APL using leukemic cells from *PML/RARα* transgenics in which a point mutation in the transgene impairs the binding of RA to PML/RARα (16). *In vivo* growth of this leukemia is much slower, liver or spleen invasion is not as pronounced and either RA or As_2O_3 have modest anti-proliferative effects in the absence of significant differentiation (see **Figures 4A, 4D and 4E**).

Spleen-derived leukemic blasts (10^7) were serially passaged in syngenic FV/B mouse (6 weeks old, weighting 20 g), as previously described (14). Both RA-sensitive leukemia (strain 935) or RA-resistant ones (strain 4048 (16)) were used. Animals were treated according to institutional guidelines. All experiments involving mice were repeated between 2 and 8 times, usually with two mice in each treatment arm. Alzet pumps (0.5 µl/h, Cupertino, CA) were loaded with 8-Cl-cAMP (20 mg/ml) and implanted subcutaneously on the back of treated mice. Aminophylline, a soluble precursor of theophylline, was injected intraperitonealy (100 µl/day of a 25 mg/ml solution, Renaudin, France). All-trans retinoic acid (Innovative Research of America, Sarasota, FL) and As_2O_3 treatments, autopsies and cellular or tissue analyses were performed as previously described (14). For Western-blot, a p21 monoclonal antibody (Pharmingen, San Diego, CA) was used at a 1/500 dilution. Dosage of plasma 8-Cl-cAMP was performed by HPLC using a C18 column (Chromosep Inertil 5 ODS3) with a 15% methanol/50 mM pH 5.85 phosphate buffer as a mobile phase and U.V. detection at 254 nm.

Animals bearing established leukemia were treated with 8-Cl-cAMP, As_2O_3, RA or combinations of these drugs and sacrificed 1 to 7 days post-treatment. Continuous 8-Cl-cAMP infusions allowed significant plasma concentrations to be reached (1 µM on average at day 3). Despite its toxicity, this compound induced major anti-leukemic effects in both RA-sensitive and RA-resistant APLs, assessed by the spleen weight (Figures 1A, 1B), liver infiltration (**Figures 1C-1F**) or marrow infiltration (Figures 1G-1J). Differentiated cells were consistently observed in the marrow after 7 days of treatment in RA-resistant APL (**Figure 1F**), and often found in RA-sensitive APL (Figure 1D). In leukemic cells infiltrating the liver, a sharp reduction in the number of mitosis was observed (3% post-treatment *vs* 28% pretreatment) and, although a few condensed nuclei were seen, TUNEL assays remained negative, suggesting that 8-Cl-cAMP mainly triggers growth arrest. Yet, apoptosis was also noted in the bone marrow in some experiments (Figure 1F). Furthermore, the treatment restored normal liver architecture for RA-sensitive as well as for RA-resistant animals as evidenced by **Figures 1G-1J**. Altogether, in these RA-sensitive or RA-resistant mouse models of APL, cAMP triggers a combination of growth arrest, differentiation and apoptosis, resulting in dramatic regressions of the leukemia. Yet, in most cases, cAMP was unable to eradicate APL.

Since cAMP greatly increases As_2O_3-triggered differentiation *ex vivo* (see Example 1), the Inventors associated 8-Cl-cAMP and As_2O_3 treatments *in vivo* in a RA-sensitive mouse model of APL. With this combined treatment, the spleen, liver and bone marrow became leukemia-free between days 1 and 3, while animals treated with As_2O_3 alone retained a significant tumor burden consisting of differentiating leukemic cells (**Figures 2A, C and D**). Interestingly, erythroblasts and megakaryocytes were extremely numerous in a cell-rich marrow (**Figure 2C**), consistent with the idea that cAMP promotes regrowth of these cells. In keeping with *ex vivo* results, cAMP synergized with As_2O_3 to trigger differentiation, as was evidenced by CD11b expression on bone marrow blasts at day 1 or 2 by fluo-cytometry (**Figure 3D**), strongly suggesting that enhanced differentiation of the leukemic blasts contributes to accelerated remissions. The cdk inhibitor p21, a known cAMP target implicated both in growth arrest, differentiation and apoptosis, was sharply induced in bone marrow APL blasts (**Figure 2B**). Some synergy was also noted with RA with respect to both spleen weight and marrow differentiation, although RAs' much stronger differentiating effect dims the effect (**Figure 3A-3C**).

The stable cAMP derivative used here induces massive diuresis, precluding any long-term use, and may further be metabolized into potentially cytotoxic nucleotide analogues (15). To ensure that the antileukemic effect indeed results from activation of cAMP signaling, the Inventors used theophylline (under its stabilized form aminophylline), a phosphodiesterase inhibitor which stabilizes pools of endogenous intracellular cAMP, in our RA-sensitive APL model. Theophylline, similar to 8-Cl-cAMP, blocked APL growth and induced some apoptosis, accompanied by non-terminal differentiation (**Figure 2E**). As expected, enhancement of differentiation was more pronounced for As_2O_3 than for RA (**Figure 2E**). Yet, there was no obvious boost in normal haematopoiesis, possibly reflecting low production of endogenous cAMP in these cells.

8-Cl-cAMP induced even more dramatic regressions in RA-resistant APLs, with some morphologically complete clearances (**Figures 1B, 1E, 1F, 1I, 1J and 4A-4I**). Unexpectedly, a major enhancement in leukemia clearance and differentiation was consistently observed when RA was combined to 8-Cl-cAMP (**Figure 4A, 4G**). Synergistic effects with As_2O_3 were also noted.

EXAMPLE 3

Theophylline induces remission in a RA- and As- resistant APL patient

With these promising results in mouse APLs, a RA/As_2O_3-resistant APL patient was offered an experimental course of combined RA/As_2O_3/theophylline therapy.

The patient gave informed consent for use of theophylline to enhance RA/ As_2O_3 differentiation. The daily treatment was with RA 45 mg/m^2 P.O., As_2O_3: 10 mg I.V., theophylline 250 mg P.O.

Previously, a month of RA/As_2O_3 association had yielded a slow decrease in bone marrow blasts, a peak of differentiating myeloid cells in the blood, but normal haematopoiesis was not restored and the leukemia reappeared 5 weeks later (**Figure 5A-5C, 5G**). With combined RA/As_2O_3/theophylline, leukemic cells underwent differentiation and normal erythroblasts rapidly appeared (**Figure 5D-5G**). The patient no longer required transfusions and platelets, white blood cells and hemoglobin levels reached sub-normal levels. The patient remained leukemia-free for 4 months and then the leukemic clone reappeared. Paradoxically with ongoing RA/As_2O_3/theophylline therapy and despite leukemia relapse, normal hematopoiesis was maintained to date (5 months since relapse), a situation highly unusual in APL where cytopenia is the first sign of relapse.

These data demonstrate that, through a combination of growth arrest, apoptosis and differentiation, *in vivo* activation of cAMP signaling is beneficial in two distinct animal models of APL, as well as in a RA/As_2O_3-resistant APL patient. The relative balance between growth arrest, apoptosis and differentiation likely depends on the dose of the compound, the microenvironment of the APL blasts (marrow *vs.* liver metastasis, for example) and their nature (RA-sensitive or RA-resistant cells). At present, it is difficult to explain the greater 8-Cl-cAMP sensitivity of RA-resistant APLs, although it might be explained by their slower growth rates. As_2O_3 was initially believed to trigger apoptosis, but *in vivo* observations (14), as well *ex vivo* data (Fig. 2) (17), now implicate differentiation as alternative mechanism (reviewed in (19)). The dramatic *in vivo* enhancement of As_2O_3 triggered differentiation by 8-Cl-cAMP further strengthens this point. Cyclic AMP-triggered growth arrest may result from induction of the cell-cycle inhibitor p21, which was previously implicated in RA-induced APL differentiation (20). Cyclic AMP enhancement of RA-, As_2O_3- or rexinoids- triggered differentiation may also result from induction of the G-CSF receptor (21). In F9 embryonal carcinoma cells, cAMP was shown to modulate RA-triggered differentiation through RARα phosphorylation (22). Since *RARα* plays a critical role in

myeloid differentiation, including in IL3 or GM-CSF response (23), such modulation of RARα signaling may also contribute to cAMP response. Therapy-resistant patients only exceptionally exhibit mutations in PML/RARα, particularly in European trials, which does not favor a direct parallelism between the cases of this patient and of the RA-resistant APL mice. The RA/8-Cl-cAMP synergy for differentiation in RA-resistant APL was unexpected. It is possible that this reflects the *in vivo* conversion of RA to rexinoids, allowing the cAMP/rexinoid triggered differentiation demonstrated in cell-lines (21). Although As_2O_3 alone triggered a modest anti-proliferative effect in the absence of significant differentiation, the RA/As_2O_3 combination triggered a minor, but reproducible, differentiation (Fig. 4 b).

In the patient, as in the APL mice, cAMP induces the rapid regrowth of normal erythroblasts and megakaryocytes. This could result from a direct positive effect on the normal progenitors, as reported *ex vivo* (24). Alternatively, APL cells secrete inhibitors of normal haematopoiesis (25, 26) whose synthesis or downstream signaling, could be blocked by cAMP. In the clinical setting, such *in vivo* stimulatory effect on normal haematopoiesis could be as important as leukemia inhibition. The low toxicity of theophylline, its ability to accelerate RA- or As_2O_3–triggered remissions favor the use of theophylline in *de novo* patients.

Cyclic AMP is active both in RA-sensitive and RA-resistant APLs. Moreover, in contrast to RA and As_2O_3, cAMP does not obviously target PML/RARα and therefore might be valuable in malignancies other that APL. Non-APL myeloid cells are also very sensitive to cAMP triggered differentiation, particularly in the presence of other differentiation inducers. Similar to histone desacetylase inhibitors, which unravel RA-induced differentiation in acute myeloid leukemia (3, 27), theophylline may greatly increase the potency of other differentiation inducers *in vivo*.

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CLAIMS

1. The use of at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives, for the preparation of a drug for the treatment of cancers.

2. The use of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives and
- at least one cell-differentiation factor or precursors or derivatives thereof and/or
- at least one apoptotic inducer

for the preparation of a drug for the treatment of cancers.

3. The use according to claim 2, of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives and
- at least one cellular differentiating factor or precursors or derivatives thereof

for the preparation of a drug for the treatment of cancers.

4. The use according to claim 2, of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP and
- at least one apoptotic inducer

for the preparation of a drug for the treatment of cancers.

5. The use according to claim 2, of:

- at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives,
- at least one cellular differentiating factor or precursors or derivatives thereof and

- at least one apoptotic inducer

for the preparation of a drug for the treatment of cancers.

6. The use according to anyone of claims 1 to 5, wherein the agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives is selected from the group comprising cAMP, 8-Cl-cAMP, 8-CPT-cAMP, 8-Br-cAMP, dibutyryl-cAMP or pharmacologically acceptable salts thereof.

7. The use according to anyone of claims 1 to 5, wherein the agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives is a phosphodiesterase inhibitor.

8. The use according to claim 7, wherein the phosphodiesterase inhibitor is selected from the group comprising methylxanthines such as caffeine or theophylline or aminophylline or isobutyl-methylxanthine, rolipram, sildenafil, vardenafil, zaprinast, or methoxyquinazoline.

9. The use according to claims 7 to 8, wherein the phosphodiesterase inhibitor is theophylline or aminophylline.

10. The use according to claim 2, wherein the cell-differentiation factor or precursors or derivatives thereof is selected from the group comprising retinoic acid, particularly all-trans retinoic acid or 9-cis retinoic acid or 13-cis retinoic acid, or pharmacologically acceptable salts thereof, vitamin A (retinol), carotene or rexinoids.

11. The use according to claim 10, wherein the cell-differentiation factor or precursors or derivatives thereof is retinoic acid, particularly all-trans retinoic acid, or pharmacologically acceptable salts thereof.

12. The use according to claim 2, wherein the apoptotic inducer is selected from the group comprising arsenic trioxide (As_2O_3) or arsenic sulfide (As_4S_4).

13. The use according to claim 2, of theophylline or aminophylline and retinoic acid or pharmacologically acceptable salts thereof, for the preparation of a drug for the treatment of cancers.

14. The use according to claim 2, of theophylline or aminophylline and arsenic trioxide or arsenic sulfide, for the preparation of a drug for the treatment of cancers.

15. The use according to claim 2, of theophylline or aminophylline, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, for the preparation of a drug for the treatment of cancers.

16. The use according to anyone of claims 1 to 15, wherein the cancers are selected from the group comprising solid tumor cancer, neuroblastoma, skin cancer, oral cavity cancer, lung cancer, mammary gland cancer, prostatic cancer, bladder cancer, liver cancer, pancreatic cancer, cervical cancer, ovarian cancer, head and neck cancer, colon cancer, germ cell cancer, leukemia, acute leukemia, acute myelocytic leukemia, acute promyelocytic leukemia, aleukemic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia.

17. Products containing 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

18. Products according to claim 17, containing 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

19. Products according to claim 17, containing 8-Cl-cAMP or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

20. Products according to claim 17, containing 8-Cl-cAMP or pharmacologically acceptable salts thereof, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

21. Products containing aminophylline or theophylline and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

22. Products according to claim 21, containing aminophylline or theophylline and retinoic acid or pharmacologically acceptable salts thereof, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

23. Products according to claim 21, containing aminophylline or theophylline and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

24. Products according to claim 21, containing aminophylline or theophylline, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, as a combined preparation for simultaneous, separate or sequential use in cancer treatment.

25. A pharmacological composition comprising as active substance 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

26. A pharmacological composition according to claim 25, wherein the active substance is 8-Cl-cAMP or pharmacologically acceptable salts thereof and retinoic acid or pharmacologically acceptable salts thereof, in association with a pharmacologically acceptable vehicle.

27. A pharmacological composition according to claim 25, wherein the active substance is 8-Cl-cAMP or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

28. A pharmacological composition according to claim 25, wherein the active substance is 8-Cl-cAMP or pharmacologically acceptable salts thereof, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

29. A pharmacological composition comprising as active substance theophylline or aminophylline and retinoic acid or pharmacologically acceptable salts thereof and/or arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

30. A pharmacological composition according to claim 29, wherein the active substance is theophylline or aminophylline and retinoic acid or pharmacologically acceptable salts thereof, in association with a pharmacologically acceptable vehicle.

31. A pharmacological composition according to claim 29, wherein the active substance is theophylline or aminophylline and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

32. A pharmacological composition according to claim 29, wherein the active substance is theophylline or aminophylline, retinoic acid or pharmacologically acceptable salts thereof and arsenic trioxide or arsenic sulfide, in association with a pharmacologically acceptable vehicle.

33. A pharmacological composition according to anyone of claims 29 to 32, in a form appropriate for the administration of about 0.36 mg/kg/day to about 14.3 mg/kg/day of theophylline or aminophylline, of about 4.5 mg/m^2/day to about 135 mg/m^2/day of all-trans retinoic acid and of about 0.014 mg/kg/day to about 0.43 mg/kg/day of arsenic trioxide.

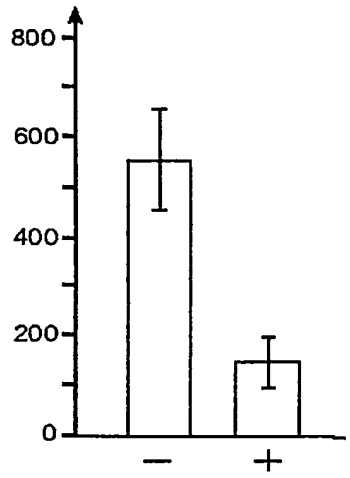


Figure 1A

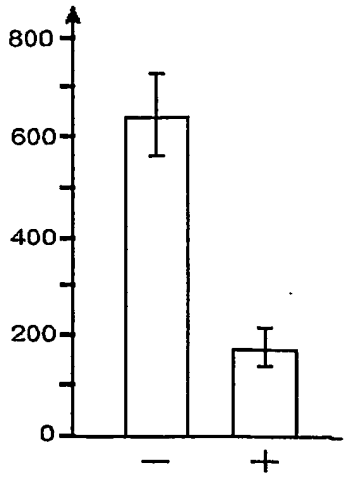


Figure 1B

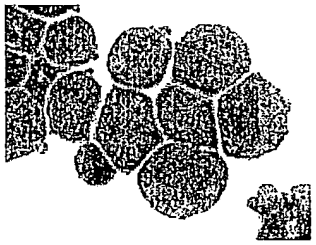


Figure 1C

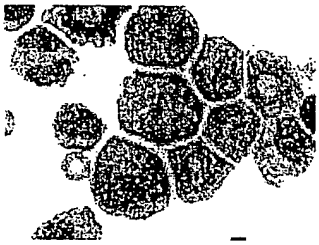


Figure 1D

Figure 1E

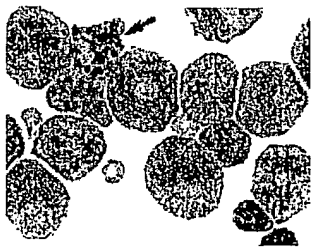


Figure 1F

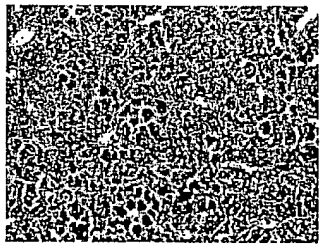


Figure 1G

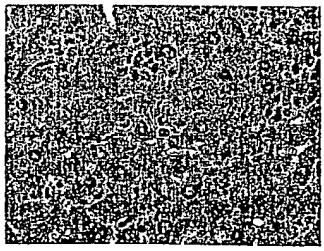


Figure 1H

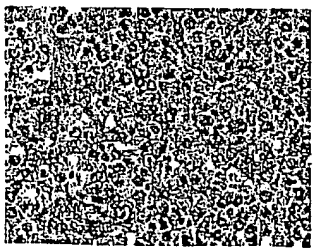


Figure 1I

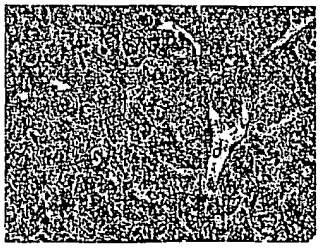


Figure 1J

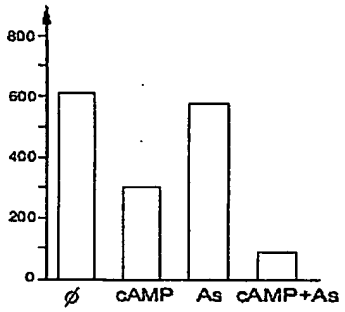


Figure 2A

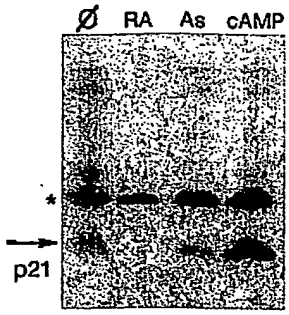


Figure 2B

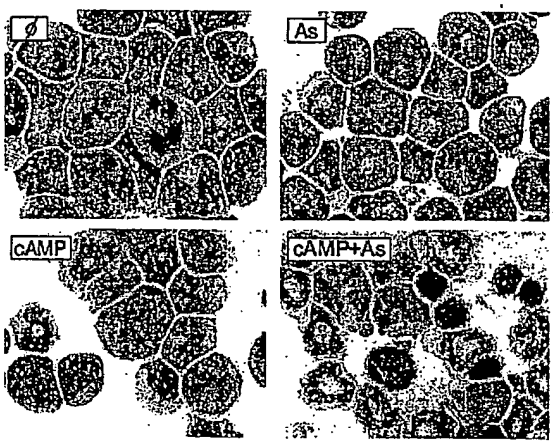


Figure 2C

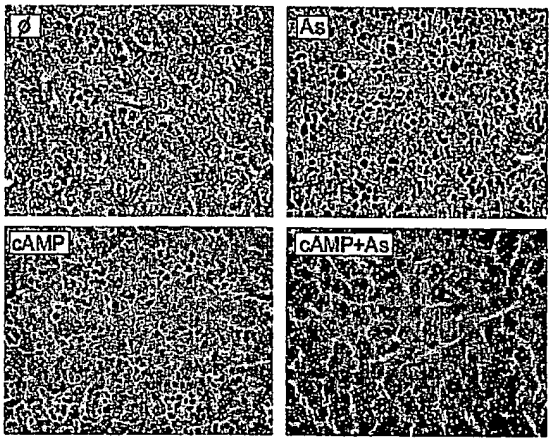


Figure 2D

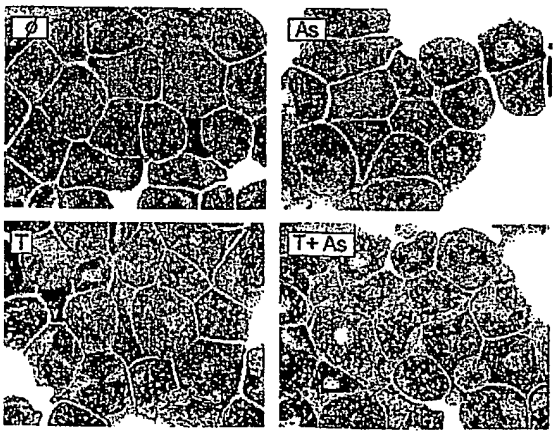


Figure 2E

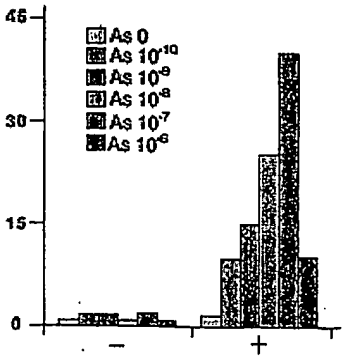


Figure 2F

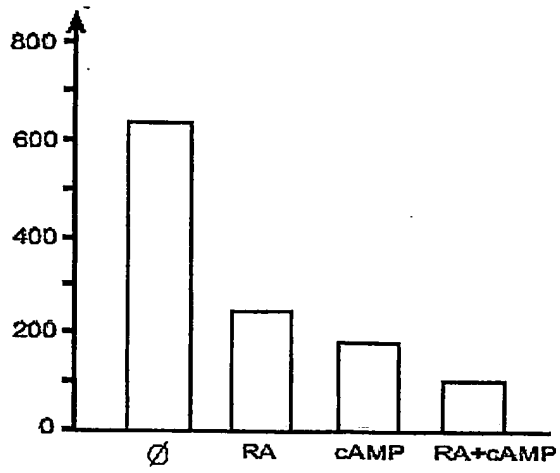


Figure 3A

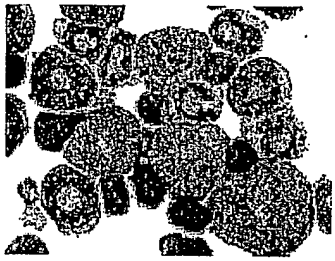


Figure 3B

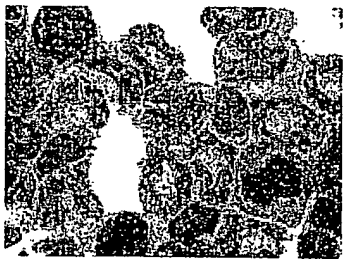


Figure 3C

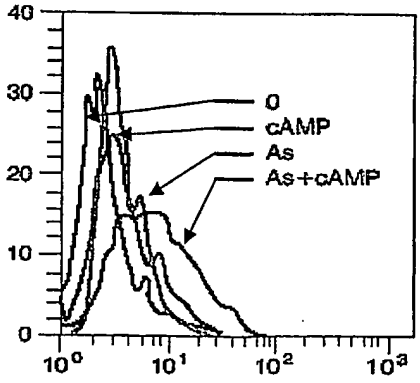


Figure 3D

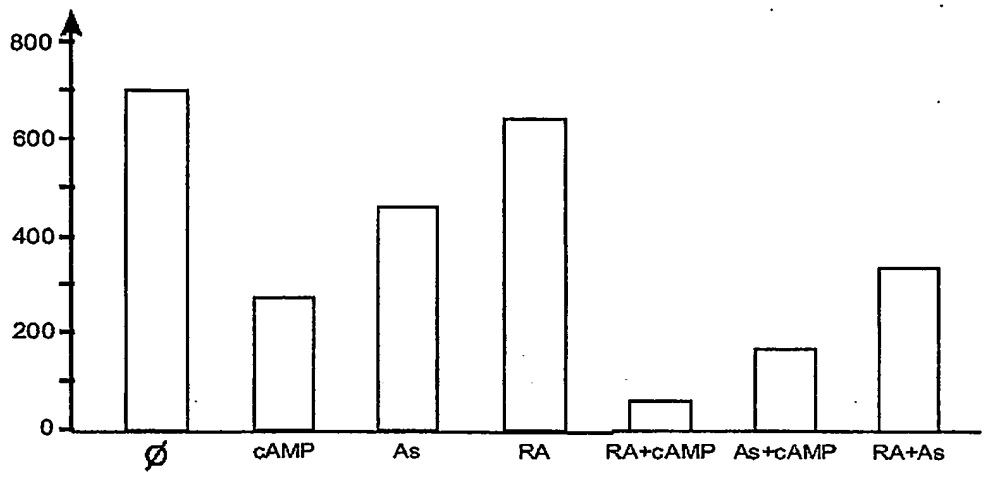


Figure 4A

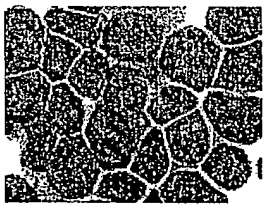


Figure 4B

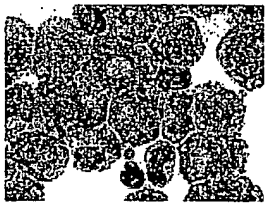


Figure 4C

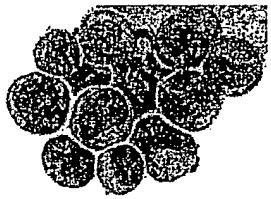


Figure 4D

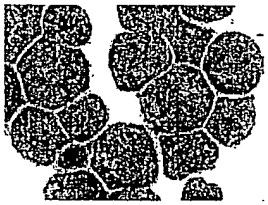


Figure 4E

Figure 4F

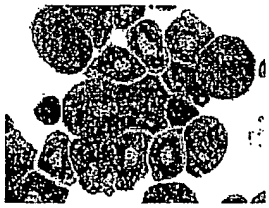


Figure 4G

Figure 4H

Figure 4I

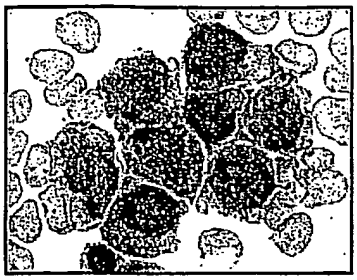


Figure 5A

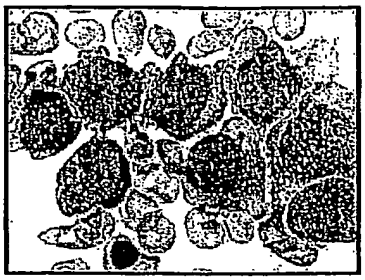


Figure 5B

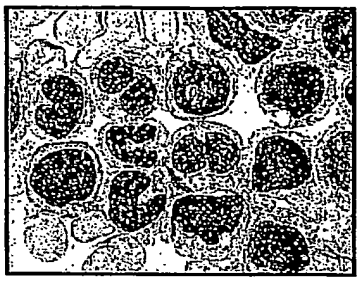


Figure 5C

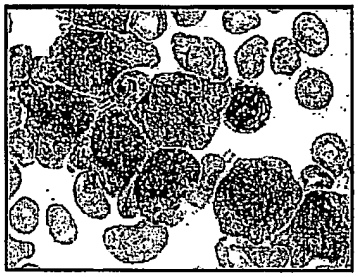
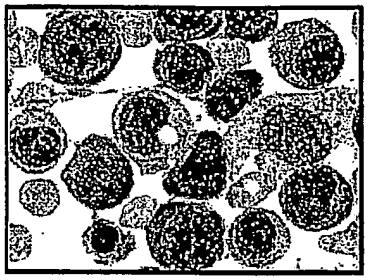


Figure 5D



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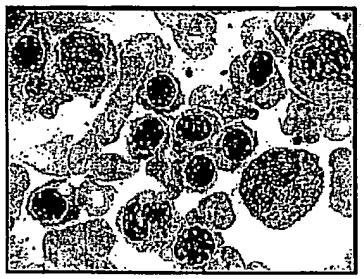

Figure 5F

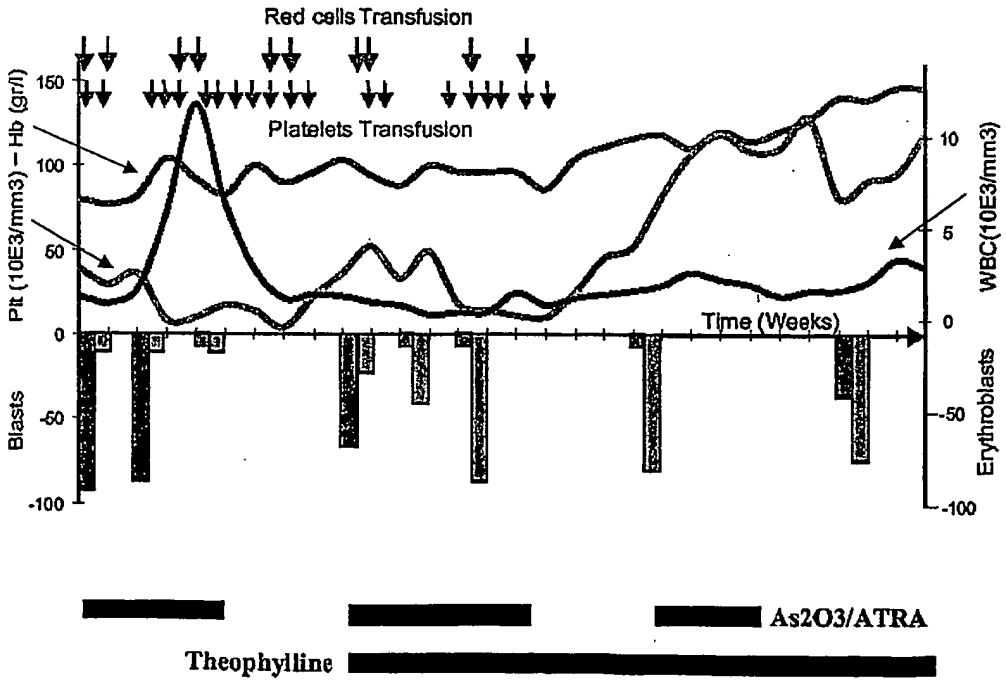

Figure 5G

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16 September 2003 (16.09.2003)

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(71) Applicants *(for all designated States except US)*: **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE** [FR/FR]; 3, rue Michel-Ange, F-75794 Paris Cedex 16 (FR). **INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM)** [FR/FR]; 101, rue de Tolbiac, 75654 Paris Cedex 13 (FR).

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(74) Agent: **GROSSET-FOURNIER, Chantal**; Grosset-Fournier & Demachy SARL, 54, rue Saint-Lazare, F-75009 Paris (FR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
2 September 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITIONS INCREASING CAMP USEFUL FOR THE TREATMENT OF CANCERS

(57) Abstract: The present invention relates to the use of at least one agent enabling to increase the cellular content of cAMP or derivatives thereof with respect to the originally present cellular content of said cAMP or said derivatives, for the preparation of a drug for the treatment of cancers.

WO 2004/026319 A3

INTERNATIONAL SEARCH REPORT

PCT/EP 03/10280

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/7076 A61K31/522 A61K31/203 A61K31/285 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GUILLEMIN MARIE-CLAUDE ET AL: "In vivo activation of cAMP signaling induces growth arrest and differentiation in acute promyelocytic leukemia." THE JOURNAL OF EXPERIMENTAL MEDICINE. UNITED STATES 18 NOV 2002, vol. 196, no. 10, 18 November 2002 (2002-11-18), pages 1373-1380, XP002267247 ISSN: 0022-1007 the whole document ----- -/--	1-3, 5-11,13, 15-18, 20-22, 24-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the International search	Date of mailing of the international search report
19 January 2004	08. 06. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

PCT/EP 03/10280

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/64260 A (INST NAT SANTE RECH MED ;UNIV PASTEUR (FR); CENTRE NAT RECH SCIENT) 2 November 2000 (2000-11-02) page 12, line 24 - page 20, line 20 page 31, line 28 - page 32, line 2	1-3,6, 10,11, 16-18, 25,26
Y	claims 1,2,9,11,19,22,24,30	1-3,5,6, 10-12, 16-18, 20,25-28

X	SRIVASTAVA R K ET AL: "Synergistic effects of retinoic acid and 8-Cl-cAMP on apoptosis require caspase-3 activation in human ovarian cancer cells." ONCOGENE. ENGLAND 4 MAR 1999, vol. 18, no. 9, 4 March 1999 (1999-03-04), pages 1755-1763, XP002267248 ISSN: 0950-9232 abstract * See page 1756, column 1: "results" * * See page 1761 column 2, paragraph 3 * figures 1,2	1-3,6, 10,11, 16-18, 25,26

X	SRIVASTAVA R K ET AL: "Synergistic effects of 8-chlorocyclic-AMP and retinoic acid on induction of apoptosis in Ewing's sarcoma CHP-100 cells." CLINICAL CANCER RESEARCH: AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. UNITED STATES MAR 1998, vol. 4, no. 3, March 1998 (1998-03), pages 755-761, XP001157237 ISSN: 1078-0432 abstract figure 2; table 1 * See results: page 757-759 * * See discussion: page 760, last paragraph *	1-3,6, 10,11, 16-18, 25,26

X	SRIVASTAVA R K ET AL: "Synergistic effects of 8-Cl-cAMP and retinoic acids in the inhibition of growth and induction of apoptosis in ovarian cancer cells: induction of retinoic acid receptor beta." MOLECULAR AND CELLULAR BIOCHEMISTRY. NETHERLANDS JAN 2000, vol. 204, no. 1-2, January 2000 (2000-01), pages 1-9, XP009024119 ISSN: 0300-8177 the whole document	1-3,6, 10,11, 16-18, 25,26

	-/--	

INTERNATIONAL SEARCH REPORT

PCT/EP 03/10280

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/04541 A (SKULNICK HARVEY I ;ABRAHAM IRENE (US); UPJOHN CO (US); ARISTOFF PA) 3 March 1994 (1994-03-03) page 29, line 17 page 30, lines 26,27 claims 25,29	1
A	CHENG L ET AL: "CHARACTERISATIONS OF TAXOL-INDUCED APOPTOSIS AND ALTERED GENE EXPRESSION IN HUMAN BREAST CANCER CELLS" CELLULAR PHARMACOLOGY, STOCKTON PRESS, XX, vol. 2, no. 6, November 1995 (1995-11), pages 249-257, XP008013734 ISSN: 1351-3214 the whole document	1
X	US 5 880 153 A (NEUMAN TOOMAS ET AL) 9 March 1999 (1999-03-09) column 9, lines 31-34 column 10, lines 28-33	17,18,25
Y	FR 2 782 010 A (INST VAISSEAUX ET DU SANG) 11 February 2000 (2000-02-11) the whole document	1-3,5,6, 10-12, 16-18, 20,25-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/10280

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1,2,3,5,6,10,11,12,16,17,18,20,25,26,27,28, (partial)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: -

In view of the expression " an agent enabling to increase the cellular content of cAMP or derivatives thereof", and the expression "cell differentiation factor", present claims 1,2,3,5 relate to an extremely large number of possible compositions. In fact, the claims contain so many options, variables, possible permutations and provisos that a meaningful search of the claims impossible. Consequently, the search for the first invention has been carried out for those parts of the application relating to compositions comprising cAMP and other nucleoside derivatives as the ones listed in claim 6 and retinoic acid derivatives as claimed in claim 10.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: Claims: 1, 2, 3, 5, 6, 10,11,12, 16,17,18, 20, 25, 26, 27, 28, (partial)

Pharmaceutical compositions comprising:
a) at least one agent increasing the cellular content of cAMP, such agent being a nucleoside derivative (especially the ones disclosed in claim 6).
b) at least one cell-differentiation factor (especially a retinoid)
c) optionally, one apoptotic inducer,
and their use in relation to the treatment of cancer.

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2. claims: 1, 2, 3, 5, 7, 8, 9, 10, 11,12, 13, 14, 15, 16, 21, 22, 23, 24, 29-33 (partial)

Pharmaceutical compositions comprising:
a) at least one agent increasing the cellular content of cAMP, such agent not being a nucleoside derivative, and preferably being a phosphodiesterase inhibitor (a methylxanthine in particular).
b) at least one cell-differentiation factor (especially a retinoid).
c) optionally, one apoptotic inducer
and their use in relation to the treatment of cancer.

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3. claims: 4, 14, 19, 23 (complete); 1, 2, 12, (partial)

Pharmaceutical compositions comprising:
a) at least one agent increasing the cellular content of cAMP
b) one apoptotic inducer (As203 or As404 in particular)
and their use in relation to the treatment of cancer.

INTERNATIONAL SEARCH REPORT

PCT/EP 03/10280

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